A CONTRIBUTION TO THE BACTERIOLOGY OF DIPHTHERIA

Being the Thesis

of

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for the Degree of Doctor of Medicine.

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During the last 25 years or so the Bacillus of Diphtheria, first described by Klebs, and almost simultaneously cultivated by Loffler, has been the subject of much interesting and instructive investigation and discussion; particularly has this been so during the last five or six years, a period which marks a great advancement in bacteriological methods.

Notwithstanding all that has been done, and all the useful information that has been the result - much still remains to be decided and to be investigated. Different observers, working apparently with the utmost care and precision, have arrived at different results, even though working along the same lines: this undoubtedly must be in a great measure due to some slight differences in methods, in preparations, etc. that is, without a standard for uniformity, allowing always therefore too greatly the personal element of the investigators to enter into the results achieved.

In the minds of many eminent investigators,

there is still doubt as to whether the pseudo-bacillus described by Hoffmann in 1888, and now generally known as "Hofmann's Bacillus",(as it will hereafter be referred to in this paper) is or is not, a modified form of the true diphtheria bacillus, (referred to hereafter as the Loffler Bacillus). In the minds of many others, however, there is no doubt - some stating positively that the two are simply different forms of the one organism, and some on the contrary stating that they are two separate and distinct organisms.

Chief among those considering the two as varieties of the one organism are Hewlett and Knight - (Transactions of Br Instit. of Preventive Med. 1st Series 1897). These investigators considered that in one case they had converted the Hofmann's Bacillus into the Loffler, but though they and others have repeated the experiemnts, these have not produced like results; Hewlett himself trying as late as 1904.

However, the greater number regard the two as distinct organisms, basing their opinions, on clinical and laboratory results. Among this number are Graham Smith of Cambridge, who has recently done considerable work on the subject(Journal of Hygiene Vol.IV 1904) and other publications), and Petrie (of the Gener Institute, London) who has worked on the lines of producing toxins with the bacillus of Hofmann.

With such experienced observers as these quoted, it is not likely that the opinions of a so much less experienced investigator as the writer will bear much weight, but the carefully carried out experiments of any investigator must claim the consideration of all who wish to regard the subject in all its aspects.

Thus it is that the writer has for several months past devoted his whole attention to conducting the investigations which form the subject matter of this paper.

The whole of the work has been carried out in the Bacteriological Laboratory of the University

of Birmingham. The illustrations have been effected by Mrs Lewis Graham, (of 45 Newhall St, B'ham) . This artist had previously had no practice in the drawing of bacteriological specimens, and she has drawn these directly from the microscope unaided by verbal description, having drawn on an enlarged scale, exactly what she has seen down the microscope; this in the writer's opinion has been a great factor of accurate impressions, where photography, which had been tried, had failed to bring out satisfactorily the features desired to be emphasized. A scale was given to the artist that she might know the relative value of the writer's terms "Long", "medium", and "short". Although the diagrams are much magnified when compared with the actual films, the writer's "long" corresponds with 5 or 7 ~~xx xxxxxx~~ microns, "medium" with 3 to 5 microns, and "short" 2 to 3 microns. In most of the cases no attempt has been made to depict the arangement of the bacilli.

In case No.3 a biological test has been performed, and this was carried out by C.J. Lewis Esq.,(M.D.Edin.) of the Birmingham University staff, the writer not being licensed.

The object of these investigations has been, Firstly, to make observations on the change in morphological type during the stay in the disease or convalescent throat, of the Loffler bacillus, in cases of clinical diphtheria, and by isolating the organisms in these cases to grow them on various sugars and other carbohydrates, etc., to see whether any constant change occurred with these in their re-action to the media, corresponding with the change in type of the organism, or to see whether similar types produced from different cases gave constantly different re-actions to the various media.

At the same time any proximation to the Hofmann type was to be particularly noted.

Secondly. To select cases commencing with the Hofmann bacillus and follow these out, seeing, where the

Loffler bacillus could be excluded from the very onset, whether the Hofmann bacillus merged into the true diphtheria, which, it is stated by some, does occur.

With regard to the first class of cases i.e. those with clinical diphtheria and giving the Loffler bacillus from the commencement, the greatest care has been taken in the selection of suitable cases. On continued work in the bacteriological laboratory, one notices that in most cases as soon as the Loffler Bacillus has been found no further search has been made for the Hofmann bacillus. In the present research, however, several films from the same swab-culture have been thoroughly searched so that the presence of the Hofmann bacillus might with safety be excluded, the presence of which at the beginning of a case would of course render useless any conclusions arrived at if the Hofmann bacillus appeared at the end of the case.

And so in the second class of cases i.e. primarily

the Hofmann bacillus, has the true diphtheria organisms been excluded in those few cases observed.

To allow of the comparisions of results of the experiments performed with those of other workers on the same subject, full details are here given of the materials worked with, and the various data necessary for the purpose.

MEDIA.

Blood Serum. Simple ox serum rendered sterile and coagulated. Sloped tubes.

Peptone Broth. Prepared according to the formula and process given by Muir & Ritchie in their "Manual of Bacteriology".

Blood Agar. The ordinary agar medium poured into Petri plates and smeared with human blood.

Glucose Gelatine. The usual gelatine medium with 1% of glucose added.

Sugars, etc. The broth to which the sugars, etc. were added (all 1% except glycerine which was 6%) was muscle-sugar-free, this because it has been observed by other investigators that the results are not so uniform where much muscle sugar is present, as it is so readily fermentable.

The serum water medium of Hiss (ox serum 1, water 3,) as used by Graham Smith, was tried, but up to the present the writer has not been quite successful in its satisfactory preparation.

PROCESS.

The first cultures from the swabs were made on ordinary sterile sloped blood serum tubes, and these were incubated for 20 to 24 hours at 36 to 37 C.

For isolation the following method was found most effective. From the first blood serum culture a suspicious colony was taken on to the point of a

platinum needle, this colony was washed off into a small tube containing about .5 C C. of sterile normal saline solution: the needle was then streaked across a slide. Several colonies were ~~thus~~ taken from each culture, a series was thus made of individual colonies in sterile saline, each of these having a corresponding streak on the slide, which was then stained; and if a pure colony of the required organism were present (shown by pure streak on slide) a loopful of the corresponding saline emulsion would be taken and spread on the serum slope or on to a blood agar plate, this incubated at 36°- 37°C. and a pure growth obtained. Of this in the earlier cases an emulsion was made, and the sugars inoculated straight away, but finding in some strains that the growth was weak for a day or two, evidently until the bacillus got used to the new medium, cultures were made in simple peptone broth and after a day or two's incubation these were transferred to the various media. These were incubated at 37°C. for 7 days and note of the changes was made

daily and recorded.

Films were made each time a swab was sent in for examination, and in many cases these had to be written for for "repeats". The films were those from the culture from the swab and where possible, particular colonies were picked out from the general growth. These were stained with Thionin Blue, Gram, and Neisser. In all cases the re-action to these stains was noted in the particular description of the case.

Secondly films were made from all the pure cultures and staining re-actions also noted.

For some cases Loffler's Methylene Blue was used, but occasionally it was found that the polar bodies stained so deeply that it was very difficult and sometimes impossible to tell these from cocci.

In reporting on the following cases as much of the clinical history as could be obtained has been recorded; this more for the sake of comparision with future records than for any discussion in this paper, seeing that the number of cases is rather small for

clinical comparisions; one or two refernces, however will be made to these data after having given the report of the cases individually.

CASE I.

Male, Age 22.

Treatment: (a) with local antiseptics till one week after membrane had disappeared.

(b) Antitoxins 4.000 units.

Hygiene. Good.

Swabs always taken from throat.

In this case the organism was isolated four times and grown on the media. Swabs were received 7 times, the first 5 being positive, at the 3rd, 21st, 24th, 27th, & 31st days from clinical onset of disease, and the last twice negative representing 35th and 51st days.

The membrane had disappeared several days before the second report was made, and thus the organism remained in throat for a period of at least 12 days after disappearance of membrane, but on the occasion of 4th report (27th day) the number of cocci was as great as that of the Bacillus Diphtheriæ. In the 5th culture the B. Diphtheriæ were extremely few and it was

with great difficulty and care that the organism was isolated.

THE ORGANISM.

Up to the 24th day there was no variation in form in the swab culture. (Fig.I.) Various shapes presented themselves, the majority being moderately long forms, irregularly staining, arangement being the typical arrangement of the bacillus of Loffler. The bacilli on the 27th day were all evenly staining, showing no segmentation, and polar bodies extremely rare: very little variety, almost all being as the predominating form in the former films, but evenly staining. (Fig.2) In the last positive film, however, where numerous cocci were present, the forms were somewhat shorter and stouter, evenly staining, though one or two showed a septum as in the bacillus of Hofmann; the organism, however, did not stain so deeply as the bacillus of Hofmann, and stained well with Neisser and weakly with Gram.

In all cases the organism in pure culture

CASE I.

Fig I.

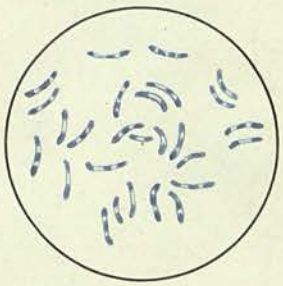


FIg.2.

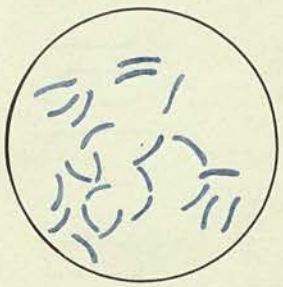
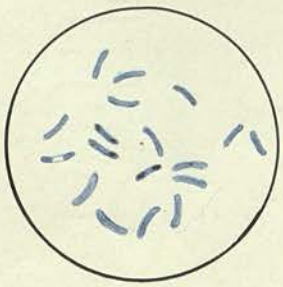


Fig. 3.



produced the same form: (Fig.3.) this being somewhat shorter and stouter than the early types, with Thionin Blue almost evenly staining, but presenting beautifully marked polar staining with Neisser.

THE CULTURE MEDIA.

Nothing of note occurred in the growth in sugars etc., these corresponding to most of the other cases; further reference will be made to these when the growth on the various cultures is discussed after the report on the several cases.

CASE I.

Day of Disease :	3	21	27	31
Dextrin	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
Levulose	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
Glucose	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
Saccharose	0000000	0000000	0000000	0000000
Maltose	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
Lactose	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
Galactose	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
Rhamnose	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
Mannose	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
Raffinose	0000000	0000000	0000000	0000000
Syringin	0000000	0000000	0000000	0000000
Salicin	0000000	0000000	0000000	0000000
Mannite	0000000	0000000	0000000	0000000
Dulcite	0000000	0000000	0000000	0000000
Adonite	0000000	0000000	0000000	0000000
Sorbit	0000000	0000000	0000000	0000000
Glycerin	0000000	0000000	0000000	0000000
Glucose Gelatin	~~0000000~~	~~0000000~~	~~0000~~ Usual growth.	
Glucose Bile S.	0000000	0000000	0000000	0000000
Lactose Bile S.	0000000	0000000	0000000	0000000
Neutral Red	Growth, but no change.			

0 = no growth with acid production
X = growth with acid production.
? = doubtful or weak acid reaction.
} throughout the paper.

N.B. Each column represents an isolation - and each X or O - the reaction on each of the seven days recorded.

CASE II.

Female Age 11.

Treatment : (a) locally formamint tablets

(b) antitoxin 4.000 units

Hygiene : Good.

Swabs all taken from throat.

Swabs were examined 5 times, the last time being a special convalescent swab, 76 days after clinical commencement, and gave very poor growth (cocci only). The first two swabs (2nd and 15th days) only were positive. Membrane disappeared about ninth day and the organism persisted till 15th day but was not present in swabs on 23rd, 30th, or 76th days. In that of the 23rd and 30th days copious growths of Staphylo strepto-cocci were present; these were not, however, present in earlier swabs.

THE ORGANISM.

There was no change in morphology or in staining re-actions of the organism when present in swab culture, both staining with Neisser well, with Gram, and with

Thionin Blue. Showing forms of moderate length and thickness with the usual arrangement. (Fig.4.)

In this strain, in pure culture, both times the culture showed many clubed forms (usually described as involution forms) staining somewhat deeply at the thickened end and faintly at the tapering end; and a second and more numerous variety of irregularly beaded form (Fig.5.) Both sub-cultures produced the same type of organism.

The CULTURE MEDIA.

The only special feature here lay in the weakness of growth in several media on the 1st day or two, this, as has been before remarked, was corrected in subsequent growth by getting the organism accustomed to the medium before putting it into the sugars.

CASE II.

Fig.4.

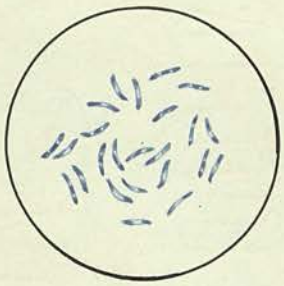
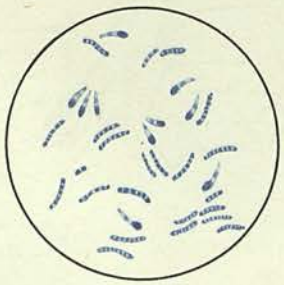


Fig.5.



CASE II

Day of Disease	2nd	15th		
Dextrin	XXXXXXX	XXXXXXX		
Levulose	XXXXXXX	XXXXXXX		
Glucose	XXXXXXX	XXXXXXX		
Saccharose	0000000	0000000		
Maltose	XXXXXXX	XXXXXXX		
Lactose	XXXXXXX	XXXXXXX		
Galactose	XXXXXXX	XXXXXXX		
Rhamnose	XXXXXXX	XXXXXXX		
Mannose	XXXXXXX	XXXXXXX		
Raffinose	0000000	0000000		
Sryingin	0000000	0000000		
Salicin	0000000	0000000		
Mannite	0000000	0000000		
Dulcite	0000000	0000000		
Adonite	0000000	0000000		
Sorbit	0000000	0000000		
Glycerin	0000000	0000000		
Glucose Gel.	Usual growth.			
Glucose Bile S.	0000000	0000000		
Lactose Bile S.	0000000	0000000		
Neutral Red	Growth but no change.			

CASE III

Female Age: 46.

Treatment : (a) local antiseptics, Potass.Chlor., weak corrosive, "Antitoxin paint" etc.,

(b) Antitoxin 4.000 units

Hygiene : Very good.

All swabs taken from throat.

This case presented the most interesting features of any of the cases under consideration: the following are briefly the clinical notes.

The patient was exposed to infection on a certain date; ten days later she complained of "sore throat": let this be regarded as the date of clinical onset. Two days later a number of white patches were seen on both tonsils and uvula, temperature 102°F. First swab was then taken and showed pure culture of Loffler's bacillus. Temperature lasted for about a week, and 6 days after the first swab was taken the membrane had all disappeared, leaving the throat inflamed and swollen, with a covering of white somewhat

opaque, mucous. About 21 days after clinical onset the swelling of throat subsided, but the throat did not assume a "healthy" aspect until about 7 weeks from the commencement.

No nasal symptoms occurred in the course of the attack. Patient had had diphtheria 20 years ago.

Local treatment was tried in a variety of ways: Potassium Chlorate gargle, Perchloride of Mercury painting 1 in 2.000, Glycerin Acid Carbolic, "a paint of antitoxin", Iodine spray with carbolic acid, and lastly a paint of 1% formalin and gargle of Potass. permang..

The disappearance of the B. Diphtheria from the throat occurred shortly after a complete change of surroundings, patient being removed to a different room. The organism persisted in the throat for 75 days from date of first seeing the membrane, notwithstanding vigorous local treatment.

Swabs were received ten times: of these the first six and the 8th were positive, the 7th, 9th,

and 10th negative, representing the days 1, 12, 22, 30, 41, 54, and 75 of disease, positive, and 65th, 80th and 90th negative.

The ORGANISM.

Isolations were effected five times, from those of 1st, 12th, 22nd, and 41st and 75th days.

The first swab presented a pure culture of an organism of medium length, (Fig.6.) staining deeply and rather unevenly though not showing distinct polar bodies, and the faint interval between. With Neisser and Gram good typical results were obtained. The second swab showed the same, staining more lightly. The third same as second, with a fair number of cocci present also, these increased in the 4th (Fig.7), which showed shorter forms but the same in characters as second and third. The 5th was the same exactly as the 4th. The 6th shorter forms of the same, but taking the stain more deeply (Fig.8.). All these varieties stained very well with Neisser. The 7th swab was negative. The 8th contained a very weak

growth with few cocci. The bacillus in this culture presented long, thin, faintly and unevenly staining, with beaded forms (Fig.9.). This one stained very badly with Neisser, and did not retain Gram. Few organisms took on the Neisser stain at all. Swabs 9 and 10 were both negative. The last organism gave the same reactions to the various media as the other organisms in this strain, but ~~this one~~ was non-virulent to a guinea-pig of about 450 grammes. The only effect produced on the animal was to make it a little less lively on the day after inoculation. No edema and no swollen glands occurred, the animal being alive and well at least a month after inoculation.

The pure culture (Fig.10.) gave the same characteristics throughout, even with the last organism. This was more like the 2nd and 3rd of the series. Of medium length, rather faintly staining with well marked polar bodies, many of the organisms having rather a streptococcal appearance. It stained well with the usual stains and had the typical "Chinese letter" arrangements.

CASE III

Fig.6.

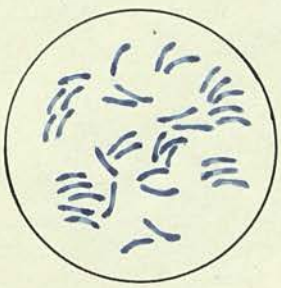


Fig.7.

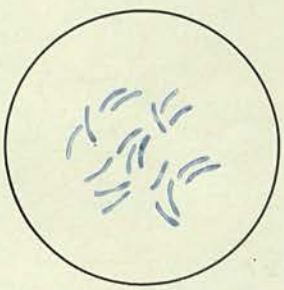


Fig.8.

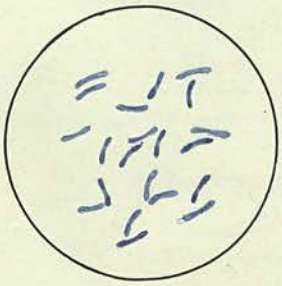


Fig.9.

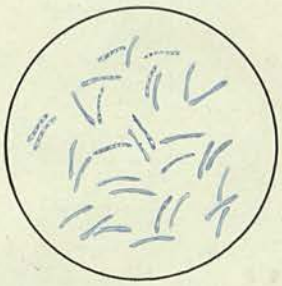
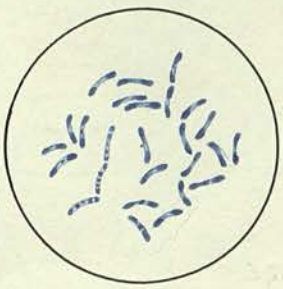


Fig.10.



The CULTURE MEDIA.

One or two differences occurred here.

Raffinose; on the 2nd and 3rd occasions this showed an acid re-action right from the first day, and on these two occasions Syringin showed no alteration the first three days, but a weak acid on the 4th and decided acid on the three subsequent days, with the other three isolations no changes occurred with Raffinose and Syringin. This variation was not due to impure culture, for several films of the new growth were examined, and growth on blood serum tried, showed pure Loffler culture.

(N.B., where variations have occurred this has always been done throughout this work.)

CASE III

Day of Disease	1	12	22	41	75
Dextrin	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
Leulose	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
Glucose	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
Saccharose	0000000	0000000	0000000	0000000	0000000
Maltose	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
Lactose	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
Galactose	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
Rhamnose	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
Mannose	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
Raffinose	0000000	??XXXXX	XXXXXXX	0000000	0000000
Sryingin	0000000	00?XXXX	000?XXX	0000000	0000000
Salicin	000000 0	000000	0000000	0000000	0000000
Mannite	0000000	0000000	0000000	0000000	0000000
Dulcite	0000000	0000000	0000000	0000000	0000000
Adonite	0000000	0000000	0000000	0000000	0000000
Sorbit	0000000	0000000	0000000	0000000	0000000
Glycerin	0000000	0000000	0000000	0000000	0000000
Glucose Gel.	Usual growth				
Glucose Bile S.	0000000	0000000	0000000	0000000	0000000
Lactose Bile S.	0000000	0000000	0000000	0000000	0000000
Neutral Red.	Growth but no change.				

CASE IV.

MALE Age: 14

Treatment: (a) Acid carbolic locally.

(b) Antitoxin 2.000 units

Hygiene: Good.

Swabs all taken from throat.

A post-scarletinal case, and one clinically presenting some features of interest, for the boy had had within three months measles, scarlet-fever, diphtheria and rheumatic-fever. The clinical manifestion of diphtheria were definite and quite in the usual run of the disease, and a fairly severe attack, owing perhaps to the peculiar series of illnesses occurring in such rapid sequence.

The organism, isolated three times, 1st, 4th, and 13th days, was in the first case a pure culture, later numerous extraneous organisms occurred; the membrane had almost disappeared when the second swab was taken at the end of the 4th day, but the bacillus was present on the 13th day, though not on the 20th, nor on the 43rd after onset.

The ORGANISM.

On the first two occasions the bacillus was very unlike the usual forms of the Loffler bacillus. It was a short,thick bacillus, (Fig.11) with a clear septum in the middle, making it look almost like a diplo-bacillus, with slight curve present; with Neisser, however, it presented a perfectly typical appearance, the blue circular or oval bodies being well defined, and often one or two other darkly stained bodies appeared along the course of the bacillus. The arrangement was as usual with the diphtheria bacillus, In the 3rd swab, the organism was longer, thinner, fairly evenly staining, but some taking on stain very poorly (Fig.12). There were present in this, as in the 2nd swab, pneumococci and staphylococci, in fair numbers. In pure culture all three produced quite a different type, this was a thin long, unevenly staining bacillus, with typical Loffler arrangement, more of the streptococcal type (Fig.13) but with Neisser looking very much as in the swab culture.

CASE IV.

Fig.11.

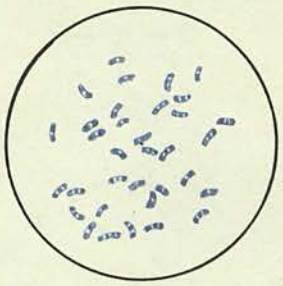


Fig.12.

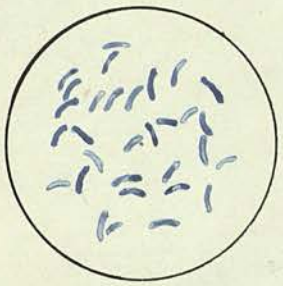
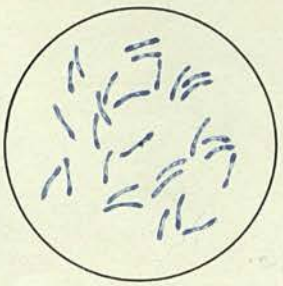


Fig.13.



The CULTURE MEDIA.

One variation occurred here. As in case III, on two occasions with Raffinose, acid was produced on the 3rd or 4th days, but only weakly; on the third occasion no such re-action was produced.

CASE IV.

Day of Disease	1	4	13
Dextrin	XXXXXXX	XXXXXXX	XXXXXXX
Levulose	XXXXXXX	XXXXXXX	XXXXXXX
Glucose	XXXXXXX	XXXXXXX	XXXXXXX
Saccharose	0000000	0000000	0000000
Maltose	XXXXXXX	XXXXXXX	XXXXXXX
Lactose	XXXXXXX	XXXXXXX	XXXXXXX
Galactose	XXXXXXX	XXXXXXX	XXXXXXX
Rhamnose	XXXXXXX	XXXXXXX	XXXXXXX
Mannose	XXXXXXX	XXXXXXX	XXXXXXX
Raffinose	000?XXX	000?XXXX	0000000
Syringin	0000000	0000000	0000000
Salicin	0000000	0000000	0000000
Mannite	0000000	0000000	0000000
Dulcite	0000000	0000000	0000000
Adonite	0000000	0000000	0000000
Sorbit	0000000	0000000	0000000
Glycerin	0000000	0000000	0000000
Glucose Gel.	Usual growth		
Glucose Bile S.	0000000	0000000	0000000
Lactose Bile S.	0000000	0000000	0000000
Neutral Red	Growth but no change.		

CASE V.

Male Age 10.

Treatment: (a) locally weak antiseptics.

(b) Antitoxin 2.000 units.

Hygiene Poor.

Swabs all taken from throat.

Examination of the organism in this case was made on the three occasions on which positive swabs were received, 2nd, 9th, and 14th days; a fourth was negative on 23rd day.

No variation in morphology occurred, and no change in the staining re-actions, or cultural characteristics occurred throughout the course of disease. The membrane did not entirely disappear until after third positive swab, thus persisting for at least 14 days, which was on the occasion of the last positive swab.

The ORGANISM.

This was a comparatively short, deeply and evenly staining bacillus, mostly, but somewhat beaded and

shorter forms are frequent. (Fig.14). Staining was good with all the stains. Characteristics were the same in both swab- and subculture.

The CULTURE MEDIA.

No variations occurred here, the re-actions being the same as the majority of the organisms have given, except that Glycerine showed acid, which only two others have done.

CASE V.

Fig.14.

CASE V.

Day of Disease	2	9	14
Dextrin	XXXXXXX	XXXXXXX	XXXXXXX
Levulose	XXXXXXX	XXXXXXX	XXXXXXX
Glucose	XXXXXXX	XXXXXXX	XXXXXXX
Saccharose	0000000	0000000	0000000
Maltose	XXXXXXX	XXXXXXX	XXXXXXX
Lactose	XXXXXXX	XXXXXXX	XXXXXXX
Glactose	XXXXXXX	XXXXXXX	XXXXXXX
Rhamnose	XXXXXXX	XXXXXXX	XXXXXXX
Mannose	XXXXXXX	XXXXXXX	XXXXXXX
Raffinose	0000000	0000000	0000000
Syringin	0000000	0000000	0000000
Salicin	0000000	0000000	0000000
Mannite	0000000	0000000	0000000
Dulcite	0000000	0000000	0000000
Adonite	0000000	0000000	0000000
Sorbit	0000000	0000000	0000000
Glycerin	XXXXXXX	XXXXXXX	XXXXXXX
Glucose Gel	Usual growth		
Glucose Bile S.	0000000	0000000	0000000
Lactose Bile S.	0000000	0000000	0000000
Neutral Red.	Growth but no change.		

CASE VI.

Female. Age: 3.

Treatment : (a) Locally Formamint tablets

(b) Antitoxin 2.000 units.

Hygiene : Poor.

Swabs taken from throat, though nose and ear also were affected at the time.

This was a somewhat peculiar case. The doctor's report given to writer was to the following effect : Child was brought to him with discharging ear, and profuse nasal discharge, nothing in the throat, (which was examined). Four days later patient was again brought, showing same condition, but this time a small patch was noticed on the uvula and on one tonsil; it was from these patches that the swabs were taken in the first instance, but these cleared up on treatment and when the last swab was taken, at the writer's request, all clinical manifestations had disappeared. Slight nasal speech occurred a few weeks later. What little antitoxin was given, was given by mouth.

Swabs were sent in altogether on 4th, 8th, 12th 16th, and 28th days, from the throat, one swab each from aural and nasal discharges taken by writer towards the end of attack, proved negative. Isolations were made from three positive swabs, 4th, 8th, and 12th days.

The ORGANISM.

The first swab presented mostly short thick forms rather lightly staining, with polar bodies and irregular outline, a few longer forms appeared with the same staining (Fig.15); there appeared to be no definite arrangement among the bacilli in the culture, but in the subculture typical arrangement was to be noticed. The first swab culture was almost pure, but in the two succeeding ones each showed a greater growth of staphylo and streptococci. The forms in the latter swabs were thinner, longer, and fainter staining,(Fig.16) In pure culture these became exactly as the ones of the first culture. (Fig.17.)

CASE VI

Fig.15

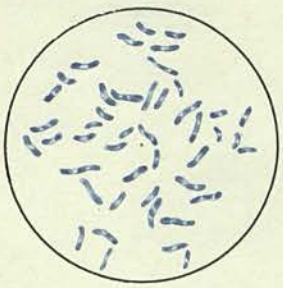


Fig.16.

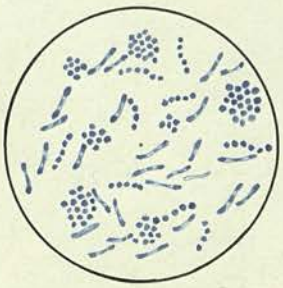
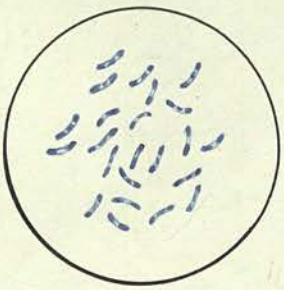


Fig.17.



The CULTURE MEDIA.

One variation only occurred here a weak acid re-action was produced in Raffinose in the first swab culture, but not in any succeeding one.

CASE VI.

Day of Disease	4	8	12
Dextrin	XXXXXXX	XXXXXXX	XXXXXXX
Levulose	XXXXXXX	XXXXXXX	XXXXXXX
Glucose	XXXXXXX	XXXXXXX	XXXXXXX
Saccharose	0000000	0000000	0000000
Maltose	XXXXXXX	XXXXXXX	XXXXXXX
Lactose	XXXXXXX	XXXXXXX	XXXXXXX
Galactose	XXXXXXX	XXXXXXX	XXXXXXX
Rhamnose	XXXXXXX	XXXXXXX	XXXXXXX
Mannose	XXXXXXX	XXXXXXX	XXXXXXX
Raffinose	0000000	0000000	0000000
Syringin	0000000	0000000	0000000
Salicin	0000000	0000000	0000000
Mannite	0000000	0000000	0000000
Dulcite	0000000	0000000	0000000
Adonite	0000000	0000000	0000000
Sorbit	0000000	0000000	0000000
Glycerin	0000000	0000000	0000000
Glucose Gel.	Usual growth.		
Glucose Bile S.	0000000	0000000	0000000
Lactose Bile S.	0000000	0000000	0000000
Neutral Red	Growth but no change.		

CASE VII

Male Age 15.

Treatment: (a) Chlorine Water

(b) Antitoxin 2.000 units.

Hygiene: Good.

Swabs all taken ffom throat.

In this case the organism persisted in the throat for a rather longer time than most of those under present consideration. Swabs were examined on 1st, 14th, 22nd, 28th, 36th, and 41st days, the last one only being negative, but the last three were full of extraneous bacilli and cocci.

The membrane persisted till, and was to a slight extent, present on the 22nd day, but disappeared shortly after this. During the time of presence of membrane, the bacilli were short, deeply and rather irregularly staining form (Fig.18) lying in bunches of threes and fours parallel, and usually in pairs lengthwise. Later on, 36th day, the organism (Fig.19) became a faintly staining one, taking Neisser poorly

and not retaining Gram, and of more scattered arrangement. In pure culture from each swab, the organism (Fig.20) was a short, deeply staining bacillus, with rather square ends, showing polar staining though not deeply marked, retaining Gram and staining well with Neisser.

The CULTURE MEDIA.

With Saccharose a weak acid production occurred after three or four days in each of the first three isolations, and in the fourth this was stronger and occurred as early as the second day. Otherwise all re-actions were as usual.

CASE VII.

Fig.18.

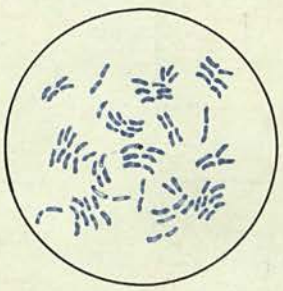


Fig.19

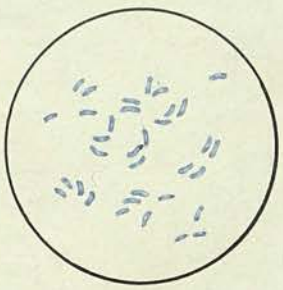
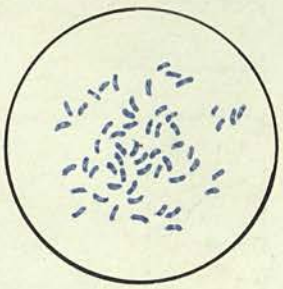


Fig.20.



CASE VII.

Day of disease:	I	I4	22	36
Dextrin	xxxxxxx	XXXXXXX	XXXXXXX	XXXXXXX
Levulose	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
Glucose	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
Saccharose	000?XXX	000?XXX	0?XXXXX	0XXXXXX
Maltose	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
Lactose	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
Galactose	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
Rhamnose	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
Raffinose	ooooooo	0000000	0000000	0000000
Syringin	0000000	0000000	0000000	0000000
Salicin	0000000	0000000	0000000	0000000
Mannite	0000000	0000000	0000000	0000000
Dulcite	0000000	0000000	0000000	0000000
Adonite	0000000	0000000	0000000	0000000
Sorbit	0000000	0000000	0000000	0000000
Glycerine	0000000	0000000	0000000	0000000
Glucose Gel.	Usual growth.			
Glucose Bile S.	0000000	0000000	0000000	0000000
Lactose Bile S.	0000000	0000000	0000000	0000000
Neutral Red	Growth, no change.			

CASE VIII

Male. Age 13

Treatment: (a) Local. Carbolic Chlorine Water gargle

(b) Antitoxin 6.000 Units.

Hygiene: Good.

Swabs all taken from the throat.

In this case clinical symptoms were well marked, and the membrane disappeared shortly after second swab (fourth day), but the bacillus was present in throat on the swab of the 15th day. Absent on the 26th and 41st days.

Swabs were sent in on 1st, 4th, 15th, 26th, and 41st days, positive on first three, negative on fourth and no growth of fifth. Isolations effected on 1st, 4th and 15th.

The growth was pure from the commencement, very few cocci being present.

The ORGANISM.

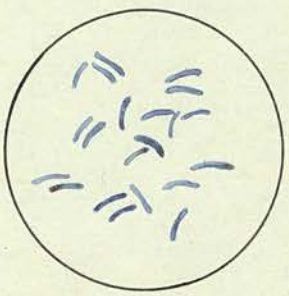
The Bacillus was of medium length throughout and alike both on swab culture and subculture (Fig.21). Staining fairly deeply and very evenly. Distinct polar bodies were seen only when stained by Neisser's method. The arrangement was the typical "Chinese letter" arrangement.

The CULTURE MEDIA.

No variation from the majority occurred in this case.

CASE VIII.

Fig.21.



CASE VIII.

Day of disease:	I	4	I5
Dextrin	XXXXXXX	XXXXXXX	XXXXXXX
Levulose	XXXXXXX	XXXXXXX	XXXXXXX
Glucose	XXXXXXX	XXXXXXX	XXXXXXX
Saccharose	0000000	0000000	0000000
Maltose	XXXXXXX	XXXXXXX	XXXXXXX
Lactose	XXXXXXX	XXXXXXX	XXXXXXX
Galactose	XXXXXXX	XXXXXXX	XXXXXXX
Rhamnose	XXXXXXX	XXXXXXX	XXXXXXX
Mannose	XXXXXXX	XXXXXXX	XXXXXXX
Raffinose	0000000	0000000	0000000
Syringin	0000000	0000000	0000000
Salicin	0000000	0000000	0000000
Mannite	0000000	0000000	0000000
Dulcite	0000000	0000000	0000000
Adonite	0000000	0000000n	0000000
Sorbit	00000000	00000000	0000000
Glycerin	0000000	0000000	0000000
Glucose Gel.	Usual growth.		
Glu.Bile S.	0000000	0000000	0000000
Lac.Bile S.	0000000	0000000	0000000
Neut. Red.	Growth , no change.		

CASE IX.

Female. Age: 13

Treatment : (a) Locally, Hydrogen peroxyd.

(b) Antitoxin 16.000 units

Hygiene: Good.

Swabs all taken from the nose.

Swabs were examined on 1st, 12, 18th, 22nd, 25th, 31st and 50th days. Of these, those taken on the 18th, 31st and 50th proved negative, and one from the throat about the 50th day also proved negative.

The history was as follows. Child was seen by doctor for a "running at the nose", which had lasted three weeks with no apparent cause, except that child seemed somewhat ill: at first diphtheria was not suspected by the doctor, but on an examination some days later, a slight membrane was seen; a swab was taken, and this showed true diphtheria bacilli. Thus the above dates are evidently not the exact dates of duration of attack, but are dates from discovery of the membrane.

The membrane could be seen in the nose as late as seventh day after discovery, and the organism was present as late as the 25th day after discovery; hence may be presumed to have been present for at least 50 days. Patient was treated consistently with Hydrogen-peroxide locally, and 16.000 units of Antitoxin, and notwithstanding the latter fact, she had slight nasopharyngeal paralysis.

The ORGANISM.

In the first swab there was a very strong staphylococcal growth and the diphtheria bacillus was spread all through the culture. It was a long thin evenly and somewhat faint staining bacillus, with rather less curve than is usual with the Löffler bacillus (Fig.22). Took Meisser well, showing polar bodies not noticeable with Thonin Blue. Gram was weakly retained.

The same character was maintained in the second swab, but the last two changed type completely, becoming more like the Bacillus Maculatus, i.e. approaching more nearly to the commonest type of Bacillus of

CASE IX.

Fig.22.

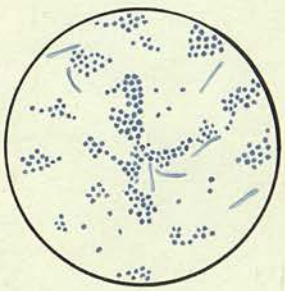


Fig. 23

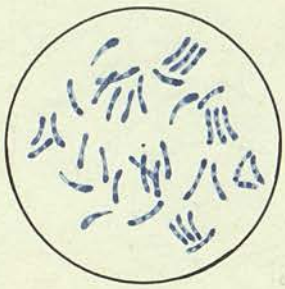
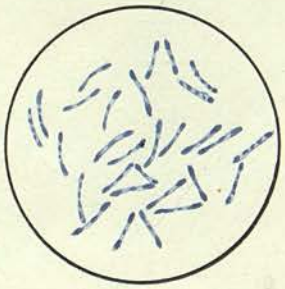


Fig.24.



Loffler: moderately long forms dotted along the course of bacillus, and showing so-called involution forms plentifully, were present, (Fig.23). In pure culture all four produced a long form not unlike the last named form, but thinner in comparison with its length. (Fig.24)

The CULTURE MEDIA.

With Galactose and Mannose in all these isolations there was no acid produced in the first two days, but by the seventh day the acid was produced as strongly as in other strains. The other media called for no remark.

CASE IX.

Day of disease:	I	I2	22	25
Dextrin	XXXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
Levulose	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
Glucose	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
Saccharose	0000000	0000000	0000000	0000000
Maltose	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
Lactose	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
Galactose	00XXXXX	00XXXXX	0XXXXXX	0XXXXXX
Rhamnose	0XXXXXXX	XXXXXXX	XXXXXXX	0XXXXXX
Mannose	0XXXXXX	00XXXXX	0XXXXXX	XXXXXXX
Raffinose	0000000	0000000	0000000	0000000
Syringin	0000000	0000000	0000000	0000000
Salicin	0000000	0000000	0000000	0000000
Mannite	0000000	0000000	0000000	0000000
Dulcite	0000000	0000000	0000000	0000000
Adonite	0000000	0000000	0000000	0000000
Sorbit	0000000	0000000	0000000	0000000
Glycerin	0000000	0000000	0000000	0000000
Glucose Gel.	Usual growth.			
Glu.Bile.S.	0000000	0000000	0000000	0000000
Lac.Bile.S.	0000000	0000000	0000000	0000000
Neut.Red.	Growth, no change.			

CASE X.

Male. Age:15

Treatment : (a) Locally.

(b) Antitoxin 10.000 units.

Hygiene: Good

Swabs all taken from throat.

A case of short duration the bacillus being present on 10th but absent on 17th day and on 31st day. Swabs were examined on 1st, 6th, 10th, 17th and 31st days. Membrane was present till 6th but not till 10th day, and the organism was almost pure culture from start to finish, and appeared same in subculture as in swab.

The ORGANISM.

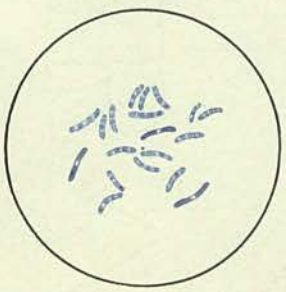
Bacilli in this rather thick, moderate length, often beaded, (Fig.25) Some with clear septum in the middle. Arrangement typical of diphtheria.bacillus.

The CULTURE MEDIA.

There was nothing of note in the sugars, the re-actions conforming to the majority of the others.

CASE X.

Fig. 25.



CASE X.

Day of disease:	1	6	10
Dextrin	XXXXXXX	XXXXXXX	XXXXXXX
Glucose	XXXXXXX	XXXXXXX	XXXXXXX
Saccharose	0000000	0000000	0000000
Maltose	XXXXXXX	XXXXXXX	XXXXXXX
Lactose	XXXXXXX	XXXXXXX	XXXXXXX
Galactose	XXXXXXX	XXXXXXX	XXXXXXX
Rhamnose	XXXXXXX	XXXXXXX	XXXXXXX
Mannose	OXXXXXX	XXXXXXX	XXXXXXX
Raffinose	0000000	0000000	0000000
Syringin	0000000	0000000	0000000
Salicin	0000000	0000000	0000000
Mannite	0000000	0000000	0000000
Dulcite	0000000	0000000	0000000
Adonite	0000000	0000000	0000000
Sorbit	0000000	0000000	0000000
Glycerin	0000000	0000000	0000000
Glucose Gel.	Usual growth		
Glucose Bile S.	0000000	0000000	0000000
Lactose Bile S.	0000000	0000000	0000000
Neutral Red	Growth, no change.		
Levulose	XXXXXXX	XXXXXXX	XXXXXXX

CASE XI

Male. Age: 8.

Treatment : (a) Locally Acid Carbolic.

(b) Antitoxin 2.000 Units.

Hygiene: Poor

Swabs all taken from throat.

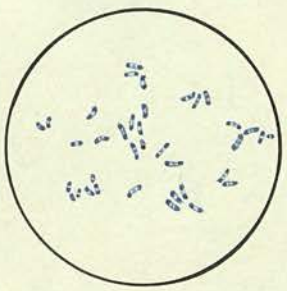
This case presented pure Loffler cultures for the whole time membrane was present i.e. up to the 10th day. Swabs were examined 1st, 6th, 10th, and 20th days. After the 10th day only a few cocci were to be found in culture.

The ORGANISM.

The bacillus throughout this case was alike both in swab and pure culture, except that the latter showed longer forms. No other change took place either morphology or staining characters; the organism was very short, a beaded form with distinct polar bodies, many young forms were present, more evenly staining than the older forms. (Fig.26) With Neisser and with Gram good results were always obtained.

CASE XI

Fig.26



The CULTURE MEDIA.

Three isolations were effected. In the first there was a weak acid produced in Saccharose, and on the 4th day a weak acid was produced in Raffinose, in the two last isolations this did not occur. Glycerin also produced acid, the other reactions were according to the usual results.

CASE XI

Day of disease	1	6	10	
Dextrin	XXXXXXX	XXXXXXX	XXXXXXX	
Levulose	XXXXXXX	XXXXXXX	XXXXXXX	
Glucose	XXXXXXX	XXXXXXX	XXXXXXX	
Saccharose	XXXXXXX	0000000	0000000	
Maltose	XXXXXXX	XXXXXXX	XXXXXXX	
Lactose	XXXXXXX	XXXXXXX	XXXXXXX	
Galactose	XXXXXXX	XXXXXXX	XXXXXXX	
Rhamnose	XXXXXXX	XXXXXXX	XXXXXXX	
Mannose	XXXXXXX	XXXXXXX	XXXXXXX	
Raffinose	000XXXX	0000000	0000000	
Syringin	0000000	0000000	0000000	
Salicin	0000000	0000000	0000000	
Mannite	0000000	0000000	0000000	
Dulcite	0000000	0000000	0000000	
Adonite	0000000	0000000	0000000	
Sorbit	0000000	0000000	0000000	
Glycerin	XXXXXXX	XXXXXXX	XXXXXXX	
Glucose Gel.	Usual growth			
Glucose Bile S.	0000000	0000000	0000000	
Lactose Bile S.	0000000	0000000	0000000	
Neutral Red	Growth, no change.			

CASE XII

Female Age 5

Treatment: (a) Locally Formamint

(b) Antitoxin 4.000 Units.

Hygiene: Fair.

All swabs taken fromthroat.

Five swabs were examined, on 2nd,5th,8th,13th and 28th days. Of these the first three were positive and of very pure growth, except that of the 8th which had many cocci present. Membrane disappeared between 5th and 8th day. After 8th day no bacilli were found.

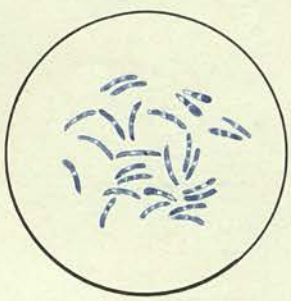
The ORGANISM.

There was no change in morphology during persistence in the throat, the organism being of medium length, staining well with theusual stains though rather lightly with Thionin Blue, specially was this so between the darker staining parts (Fig.27). In pure culture (Fig.28) there was little change except the intensity of stain being more marked, and a few clubbed forms being present.Arrangement rather scattered.

CASE XII

Fig.27

Fig.28



The CULTURE MEDIA.

The reaction obtaining with the majority of these cases, obtained with this one, with the exception of Glycerin, which produced acid.

CASE XII

Day of Disease	2	5	8	
Dextrin	XXXXXXX	XXXXXXX	XXXXXXX	
Levulose	XXXXXXX	XXXXXXX	XXXXXXX	
Glucose	XXXXXXX	XXXXXXX	XXXXXXX	
Saccharose	0000000	0000000	0000000	
Maltose	XXXXXXX	XXXXXXX	XXXXXXX	
Lactose	XXXXXXX	XXXXXXX	XXXXXXX	
Galactose	XXXXXXX	XXXXXXX	XXXXXXX	
Rhamnose	XXXXXXX	XXXXXXX	XXXXXXX	
Mannose	XXXXXXX	XXXXXXX	XXXXXXX	
Raffinose	0000000	0000000	0000000	
Syringin	0000000	0000000	0000000	
Salicin	0000000	0000000	0000000	
Mannite	0000000	0000000	0000000	
Dulcite	0000000	0000000	0000000	
Adonite	0000000	0000000	0000000	
Sorbit	0000000	0000000	0000000	
Glycerin	XXXXXXX	XXXXXXX	XXXXXXX	
Glucose Gel.	Usual growth			
Glucose Bile S.	0000000	0000000	0000000	
Lactose	0000000	0000000	0000000	
Neutral Red	Growth, no change.			

CASE XIII

Male. Age:20

Treatment: (a) Carbolic Acid. Chlorine Water

(b) Antitoxin 8.000 units.

Hygiene: Good.

Swabs all taken from throat.

Thispatient was ill for two days before sending for doctor. Membrane was then thick, and the swab showed very heavy growth of Loffler bacillus. Swabs were examined 3rd, 17th. 22nd,28th, 34th, 38th, 45th 49th days. The membrane was not present on 17th day, but the throat had a septic appearance, the swab, however, showed a variety of organisms, among which the Loffler bacilus was of very frequent occurence; few Loffler bacilli were present in the swab of 22nd day, although other organisms were plentiful. Swabs of 28th and 34th days showed few organisms at all, but the B. Loffler reappeared of the same type on 38th day, but was not seen again on 45th or 49th days.

The ORGANISM.

In swabs of 3rd, 17th, and 22nd days the bacillus (Fig.29) was a long variety, with definitely staining polar bodies, and the intervening substance very lightly stained: with Neisser, the organism stained well, with Gram weakly. In the swab of 38th day, however, a change occurred, thinner forms, somewhat longer, but less unevenly staining than the former, the arrangement in all cases was typical (Fig.30).

In pure culture the results were quite different, all types produced a shorter form more deeply staining growing in parallel lines, taking all the stains well (Fig.31).

The CULTURE MEDIA.

No special feature presented itself in this strain.

CASE XIII

Fig.29

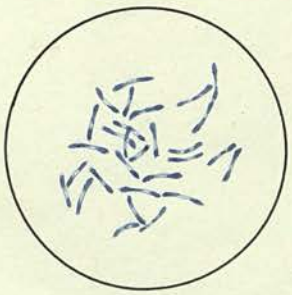


Fig.30

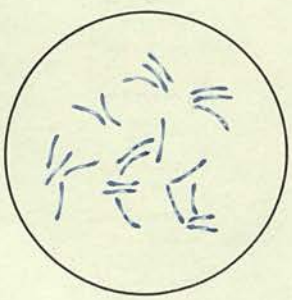
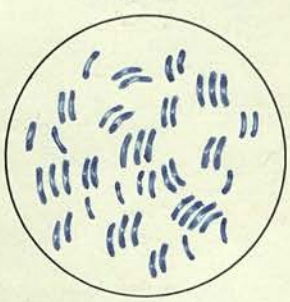


Fig.31.



CASE XIII

Day of disease	3	17	22	
Dextrin	XXXXXXX	XXXXXXX	XXXXXXX	
Levulose	XXXXXXX	XXXXXXX	XXXXXXX	
Glucose	XXXXXXX	XXXXXXX	XXXXXXX	
Saccharose	0000000	0000000	0000000	
Maltose	XXXXXXX	XXXXXXX	XXXXXXX	
Lactose	XXXXXXX	XXXXXXX	XXXXXXX	
Galactose	OXXXXXX	XXXXXXX	XXXXXXX	
Rhamnose	XXXXXXX	XXXXXXX	XXXXXXX	
Mannose	XXXXXXX	XXXXXXX	XXXXXXX	
Raffinose	0000000	0000000	0000000	
Syringin	0000000	0000000	0000000	
Salicin	0000000	0000000	0000000	
Mannite	0000000	0000000	0000000	
Dulcite	0000000	0000000	0000000	
Adonite	0000000	0000000	0000000	
Sorbit	0000000	0000000	0000000	
Glycerin	0000000	0000000	0000000	
Glucose Gel.	Usual growth			
Glucose Bile S.	0000000	0000000	0000000	
Lactose Bile S.	0000000	0000000	0000000	
Neutral Red.	Growth, no change.			

CASE XIV.

Female. Age 8

Treatment: (a) Locally, Formamint

(b) Antitoxin 4.000 units.

Hygiene: Good.

Swabs all taken from throat.

SWabs were examined on 2nd, 7th, 23rd, 27th and 33rd days, the first three had the bacillus, and the last, cocci only.

First two were almost pure in culture, the third was a weak growth with a few cocci. Membrane disappeared about the fifth day. On all three occasions the organism was of similar type, and showed a little variation on subculture.

The ORGANISM.

The prevailing form in this strain (Fig.32) was a rather tapering variety of medium length, staining faintly in middle and deepening towards the ends. The subculture media showed numerous groups of twos and threes, lying together, parallel to each other,

CASE XIV

Fig.32.

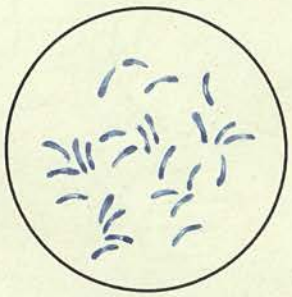
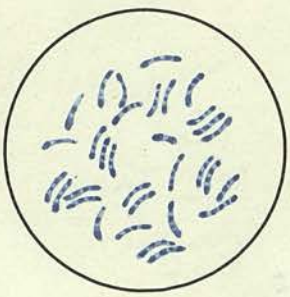


Fig. 33.



deeper staining and more irregularly (Fig.33) all forms stained well with the usual stains.

The CULTURE MEDIA.

The only feature of note in this was that acid in Lactose in the second two growths was very slow, not appearing till third day in each case, and then only weakly.

CASE XIV.

Day of Disease	2	7	23	
Dextrin	XXXXXXX	XXXXXXX	XXXXXXX	
Levulose	XXXXXXX	XXXXXXX	XXXXXXX	
Glucose	XXXXXXX	XXXXXXX	XXXXXXX	
Saccharose	XXXXXXX	XXXXXXX	XXXXXXX	
Maltose	XXXXXXX	XXXXXXX	XXXXXXX	
Lactose	XXXXXXX	OOXXXXX	OOXXXXX	
Galactose	XXXXXXX	XXXXXXX	XXXXXXX	
Rhamnose	XXXXXXX	XXXXXXX	XXXXXXX	
Mannose	XXXXXXX	XXXXXXX	XXXXXXX	
Raffinose	0000000	0000000	0000000	
Syringin	0000000	0000000	0000000	
Salicin	0000000	0000000	0000000	
Mannite	0000000	0000000	0000000	
Dulcite	0000000	0000000	0000000	
Adonite	0000000	0000000	0000000	
Sorbit	0000000	0000000	0000000	
Glycerin	0000000	0000000	0000000	
Glucose Gel.	Usual growth			
Glucose Bile S.	0000000	0000000	0000000	
Lactose Bile S.	0000000	0000000	0000000	
Neutral Red.	Growth but no change.			

CASE XV.

Male. Age: 1½

Treatment: (a) Weak corrosive locally

(b) Antitoxin 4.000 units (per os).

Swabs all from the throat. Hygiene: Poor.

Swabs were examined on 2nd, 8th, 15th, and 22nd days. First three were positive, but owing to poor growth of bacillus amongst the other organisms the third one was not isolated. Membrane had disappeared just before second swab was taken.

The ORGANISM.

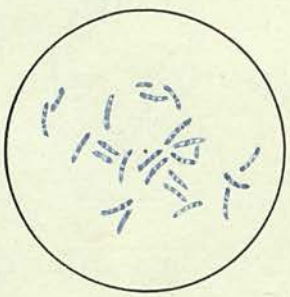
This showed the same throughout, in culture and subculture. In the latter a few shorter forms of the same kind were present (Fig.34). It was a very common type, fairly short, curved bacillus with uneven and polar staining, rather beaded, retaining the dye in Gram and staining well with Neisser. The usual arrangement existed.

The CULTURE MEDIA.

No variation occurred with the culture media.

CASE XV.

Fig. 34.



CASE XV.

Day of Disease	2	8		
Dextrin	XXXXXXX	XXXXXXX		
Levulose	XXXXXXX	XXXXXXX		
Glucose	XXXXXXX	XXXXXXX		
Sacchrose	0000000	0000000		
Maltose	XXXXXXX	XXXXXXX		
Lactose	XXXXXXX	XXXXXXX		
Galactose	XXXXXXX	XXXXXXX		
Rhamnose	XXXXXXX	XXXXXXX		
Mannose	XXXXXXX	XXXXXXX		
Raffinose	0000000	0000000		
Syringin	0000000	0000000		
Salicin	0000000	0000000		
Mannite	0000000	0000000		
Dulcite	0000000	0000000		
Adonite	0000000	0000000		
Sorbit	0000000	0000000		
Glycerin	0000000	0000000		
Glucose Gel.	Usual growth			
Glucose Bile S	0000000	0000000		
Lactose Bile S.	0000000	0000000		
Neutral Red.	Growth, but no change.			

Summary of Reactions.

CASE.	I.	2.	3.	4.	5.	6.	7.	8.	9.	I0.	II.	I2.	I3.	I4.	I5.
Dextrin	X	x	x	X	X	X	X	X	X	X	X	X	X	X	X
Levulose	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Glucose	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Sacchar.	0	0	0	0	0	0	X	0	0	0	X0	0	0	0	0
Maltose	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Lactose	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Galact.	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Rhamnose	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Mannose	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Raffinose	0	0	X 0	X0	0	0 X	0	0	0	0	X0	0	0	0	0
Syringin	0	0	0X	0	0	0	0	0	0	0	0	0	0	0	0
Salicin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mannite	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dulcite	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Adonite	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sorbit	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glycerine	0	0	0	0	X	0	0	0	0	0	X	X	0	0	0
Glu. Gel.	All produced growth along needle track.														
*Glu.Tc.B.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lac.Tc.B.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Neut.Red.	Growth, but no change in color in all.														

* "Glu.Tc. B." viz Glucose Taurocholate Broth.

With regard to the second class of cases, those exhibiting the Hofmann Bacillus from the onset, the writer found some difficulty in carrying out the work. When the presence of B. Hofmanni had been reported, the Doctor usually did not send in a second swab, these had to be continually sent for and often could not be obtained. Moreover one finds that in some cases a second swab reveals nothing but cocci, or common bacilli of the mouth.

Three good cases were examined, and each presented exactly the same features. There was no difference in any respect whatever in Morphology or staining of the bacillus from start to finish of each case, nor was there any variation in form of the three organisms being all of the usual type known as Hofmann's. With sugars, no acid was produced in any single instance.

Further details of these cases would add no interest to this paper and only unduly prolong it.

Regarding the series of True Diphtheria cases as a whole many features of interest are presented to the observer. These will be considered under the headings: "Morphology," "Cultural Reactions" and "Relations of the Bacillus Loffler and Bacillus of Hofmann," a short note on the clinical data will be added.

MORPHOLOGY

Some very striking features are to be observed under this heading. In 1900 Wesbrook Wilson and McDaniel - three American investigators - collaborating made a very elaborate classification of the types of B. Diphtheria, comprising 19 forms (published in Journal of Boston Society of Med. and Science, and since then reprinted in pamphlet form). Many others have made classifications, the types in each case varying in number; all these classifications seem to be founded on different bases. Most of the types in this investigation correspond very closely to one or other of the types of W.W. & Mc.D.

During subcultivation there appears to be a great tendency for the whole series of each strain to produce an uniform type in pure subculture.

Of the fifteen cases examined, five strains (Nos. 5, 8, 10, 11 and 15) shewed no variation in any course of cultivation, either in swab culture or pure subculture; in these, not once did the organism persist in the throat for more than 15 days, and again in not one of the cases was there any strong growth of extraneous organisms. In Strain XI there was very little irregularity of staining, but the remaining four shewed much similarity, all being more or less beaded forms.

In cases II, XII and XIV, the organism maintained its characters more or less throughout the stay in the affected part, but shewed on sub-culture a difference in type, but a difference only fairly well marked. In these cases, II persisted 15 days, XII only 8 days, and XIV for twenty-three days: With the former two many extraneous organisms were in the swabs, but this was not so in the last one.

The remaining seven cases, viz:- I, III, IV, VI, VII, IX and XIII (i.e. almost 50%) all shewed variation more or less during the stay in throat or nose. The period of stay in diseased part was respectively 31, 75, 13, 12, 36, 50 and 38 days, and every one of these shewed copious growths of extraneous organisms, chiefly staphylo-and streptococci. Again, on pure subculture, every one of these strains produced its own peculiar type. In these there was no great similarity, except that in subculture all cases except VI produced decidedly long forms.

From these facts it may be deduced:

(a) That where a strong growth of extraneous organisms occurs (in these cases it has been chiefly staphylo and streptococci). the bacillus becomes modified in its shape, not necessarily a longer form, but a different form,

though generally a longer form.

(b) That long duration of the organism in the throat is not necessary, but it contributes towards a change in the Morphology of the bacillus: and that where a strong extraneous growth occurs together with long duration in the throat, changes is almost certain to occur.

(c) That in all cases that change occurs in the Morphology, all types of one strain revert in pure subculture to one type peculiar to that strain, which may be quite unlike any of the organisms straight from swab culture: This was borne out in every case examined where change occurred, i.e. in ten out of fifteen cases.

REACTIONS WITH MEDIA

All strains produced acid with, Dextrin, Levulose, Glucose, Maltose, Lactose, Galactose, Rhamnose and Mannose.

No strain produced acid with, Salicin Adonite, Mannite, Dulcite, Sorbit, and none produced any change with Lactose-or Glucose-bile-Salt, or with Neutral Red. With gelatine stab culture the usual weak growth grew right along the track in all cases.

With Saccharose, Strain VII shewed a weak acid growth in the third isolation, and

strain XI shewed a weak acid growth in the first isolation.

With Raffinose there seemed to be some variation. With Strain IV two out of five isolations produced a weak acid reaction on the third day or so and with Strain XI in one out of three isolations a weak acid was produced on the fourth day. That is to say, (2) three strains out of fifteen produced a weak acid with this sugar, from one or more isolations.

With Syringin, on one occasion (Strain III) acid was twice, out of five isolations produced, though not on any other occasion in this series of cases.

With Lactose, growth appeared every time with acid, though not in any one case was this a marked acid, but always weak.

These results do not altogether correspond with the latest results of Graham Smith (who worked at this subject several times, the latest being 1906), but he worked with the serum-water medium of Hiss, and the writer has not yet successfully made this a suitable medium (from the process sent him by Graham Smith).

The latter found Lactose produced acid generally more readily than the writer has found it. Also with Glycerine, the writer found acid produced only in three cases out of fifteen;

Graham Smith finds it "Generally" produced.

There seems to be no change in the reactions to sugars in any of the strains, corresponding to the change of type of organism. For instance in Case III, where the organism is present 75 days in the throat the reactions on the sugars of the first type of this strain corresponded with the reactions in the last type of the same strain, and there were great differences in the two types of organism. In case IX where the organism was 50 days in the nose, and XIII, 38 days in the throat, the same observation holds good, and so in all the cases.

Again in those four cases which produced acid in Raffinose, there seems to be no common element which might reasonably be thought to account for this, nor was there in those that produced acid in Glycerine.

RELATIONSHIP BETWEEN THE BACILLUS OF LOFFLER AND BACILLUS OF HOFMANN.

With the few cases under consideration, there is not enough matter to make any emphatic statement regarding the change of one organism into the other; nevertheless, the investigations tend to add to the already large amount of evidence proving the two organisms to be distinct and separate species.

In no case did the B. Diphtheria in the 15 cases followed out, become a Hofmann, although these 15 presented many modifications; Case I. was the nearest approach, and this only in form, for the sugar reactions did not vary from the usual reactions with the true diphtheria bacillus, whereas the Hofmann bacillus produced no acid with any of the sugars.

The other variations which occurred in the bacillus when a long time in the throat, did not approach the form of Hofmann but rather shewed a tendency to longer forms. ~~With~~

With the Hofmann cases, no change occurred in the type of bacillus, but neither did the Loffler Bacillus appear whilst the former was present in the throat, nor did it follow it. These cases were only three, however, and can be of little value to put forth as evidence by themselves.

CLINICAL FEATURES

This series of cases is too limited to form the basis of argument, but it is worthy of note that Antitoxin is in general use (it was used in every case mentioned) although in many of these cases it was used in exceedingly small quantities.

The use of this does not seem to have any effect on the stay of the bacillus in the throat: nor apparently has it any effect on the alteration in type of the bacillus.

Claude R.A. Coldicutt.
Birmingham. 27th April 1909.